

A mythical beast

Increased attention highlights the hidden wonders of chimeras

On the popular US TV series *CSI*, a rapist almost walks free until crime scene investigators realize he is a chimera: the semen found at the crime scene came from his unborn twin. In an episode of *House, MD*, doctors tackle the case of a boy, a product of *in vitro* fertilization, who thinks he has been part of an alien experiment. Conflicting medical tests and the discovery of cells with a different type of DNA are not evidence of alien torture, but rather a sign of the real problem: he is a chimera. On *Grey's Anatomy*, a tumour turns out to be the testis of a vanished twin, revealing an adolescent to be a hermaphrodite—and a chimera. On *All My Children*, tests initially indicate that Emma is not Annie's daughter, though Annie swears she gave birth to the girl. A switch in the nursery? No: tissue tests from several organs show Emma is a chimera. The examples do not end there. Over the past few years, US comedies, documentaries and even cartoons have featured chimeric characters, and the condition is now beginning to appear in European programming.

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Genetics have been a common story line in police series, medical dramas and soap operas for some time. But now that DNA has become mundane, writers are turning to unusual medical conditions such as chimerism to boost their plots. Wikipedia attributes this chimera-mania to Lawrence Lessig, a law professor at Stanford University (CA, USA), who described chimerism as an underused plot device in his 2004 book

Free Culture. Within months, Hollywood responded. Ironically, although chimerism has long been thought to be rare in humans, the TV writers might have inadvertently got it right. Chimerism is not rare, but rarely discovered, because it seldom generates any observable anomalies.

A chimeric human, or human chimera, has two or more populations of genetically distinct cells that originated from different zygotes. Cells with the 'extra' genotype might be found in any part of the body, but have not yet been found mixed through all tissues of a body. The extra cell line(s) can be acquired through transplantation, transfusion or transfer of fetal cells into the circulation of the mother, but most frequently chimerism occurs spontaneously during embryogenesis. *In vitro* fertilization apparently increases the likelihood of chimerism as a side effect of the increased frequency of twinning in all pregnancies involving artificially induced ovulation. Except in the case of feto-maternal transfers, spontaneous human chimeras are di-zygotic twins whose bodies have incorporated cells of both genotypes during their embryogenesis from a single mass of cells.

A significant portion of the population might be affected by chimerism, with repercussions for transfusion medicine, transplantation and forensic science. "About one-eighth of all conceptions and about one-eighth of live births are twins—the majority of whom are born alone without a live twin. About one in eight of everybody walking around is a twin who was born single," said Charles Boklage, a developmental biologist at the Brody School of Medicine at East Carolina University (Greenville, NC, USA) who has studied chimerism for more than 25 years. For the public, the idea of carrying around parts of an unborn twin is

not easy to accept. "Look up the definition of 'chimera' in an ordinary dictionary. A major fraction of the definition has to do with something fanciful, imaginary, mythical. It has a really big 'yuck factor' for most people, to imagine a substantial fraction of us are made of cells from what might have been two people," Boklage explained.

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The frequency of spontaneous human chimerism might have been greatly underestimated, because such people are usually discovered only by chance. "When both cell lines in a chimeric individual are normal and of the same sex, there is nothing to make us investigate them. They are totally indistinguishable by ordinary observation from people with one genotype," Boklage said. "The first few [cases] reported were found in blood banks, when genotype testing showed three or four versions of each of several genes instead of the expected one or two." Even in those cases, the condition often goes unnoticed unless the second cell line is present at a proportion of 25% or more. "It has shown up when a surgical patient had a dramatic reaction to a unit of blood with a small fraction of a chimeric second line—the wrong kind of cells," Boklage said, referring to a case in which a transfusion of mixed cells caused an acute intravascular haemolysis (Pruss *et al*, 2003).

In 2001, transfusion medicine specialist Willy Flegel and colleagues at the University Hospital in Ulm, Germany, reported on a chimeric blood donor whose mixed blood had transformed the RhD antibody profiles in some transfusion recipients (Wagner *et al*,

2001). Their discovery led to changes in the policy for routine blood screening at German blood centres in 2002, and later in Austria, Switzerland and the Netherlands, and sparked discussions of possible changes in routine blood testing elsewhere in Europe and North America (Flegel, 2005).

According to Flegel, weak forms of D-positive—also called DEL—occur among D-negative people at a rate of 1:1,000 in Europeans, but 1:3 in East Asians (Flegel, 2006). “These people look like D-negative and most of them are D-negative, but some of them are expressing the D-positive antigen in a weak form which escapes standard detection techniques,” he said. “If these weak forms of D-positive are transfused to D-negative recipients, then these recipients may be immunized against the D antigen, an outcome with potentially dangerous clinical consequences. For example, if a woman is immunized to develop anti-D antibodies, this may have lethal consequences for her future pregnancies. If proper treatment is not initiated, the situation can be life-threatening for any D-positive babies.”

The Ulm group applied a new molecular test for chimerism and DEL phenotypes to more than 8,400 blood donors, which revealed one healthy man with a stable mix of 95% D-negative and 5% D-positive red blood cells (Wagner *et al*, 2001). But based on statistical forecasts, Flegel believes that this man is not the only chimeric individual among the more than 100,000 donors per year at his institution. “At the moment we cannot give a more definite or more precise number. We are still doing studies to see how frequent this situation is. But the point is even if it is only 1 in 25,000, it is a significant clinical problem,” he said, adding that the

chimeric patient can now continue to donate blood without dire consequences for recipients, owing to the new test.

Chimerism also has an impact on transplantation medicine. The prevalent view was that chimeric cells found in transplanted organs came from the recipients. But in a series of autopsies on 46 women without transplants, Marije Koopmans and colleagues from the University Medical Center in Leiden, the Netherlands, found chimeric male cells in 13 kidneys, 10 livers and 4 hearts from 23 women (Koopmans *et al*, 2005).

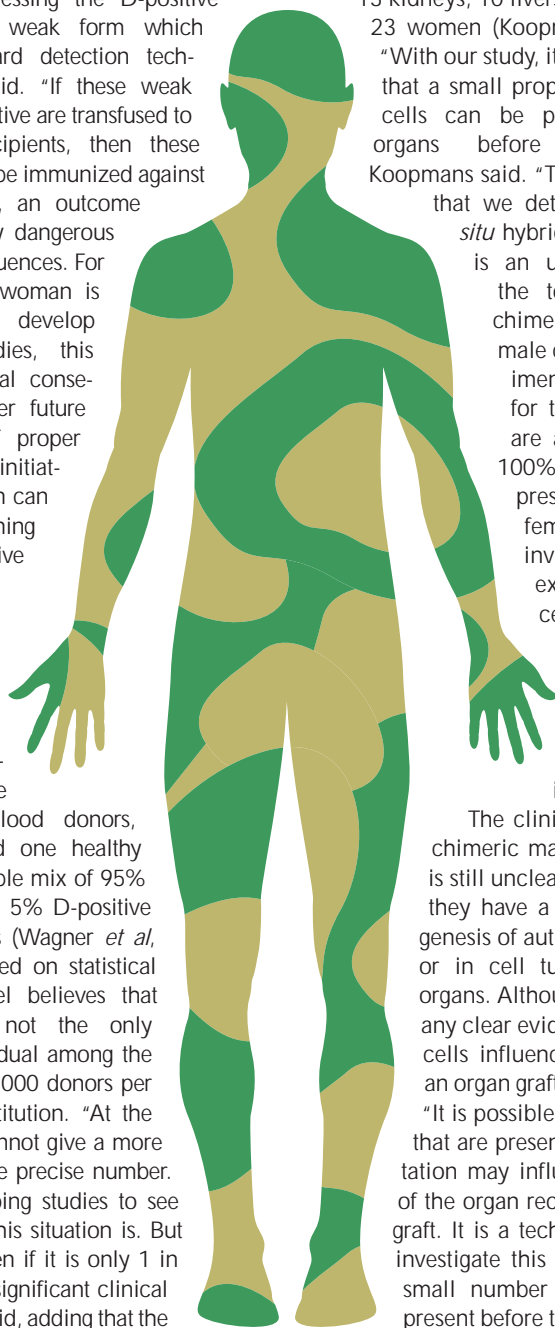
“With our study, it was demonstrated that a small proportion of chimeric cells can be present in healthy organs before transplantation,” Koopmans said. “The number of cells that we detected with our *in situ* hybridization technique is an underestimation of the total frequency of chimerism, because in male control tissue specimens, positive signals for the Y chromosome are always lower than 100%. Furthermore, the presence of chimeric female cells was not investigated, and it is expected that these cells are present as well. We had not expected such a high prevalence of chimeric cells in normal internal organs.”

The clinical significance of chimeric male cells in women is still unclear: it is possible that they have a role in the pathogenesis of autoimmune diseases, or in cell turnover in normal organs. Although there is not yet any clear evidence that chimeric cells influence the outcome of an organ graft, Koopmans noted: “It is possible that chimeric cells that are present before transplantation may influence the reaction of the organ recipient to the organ graft. It is a technical challenge to investigate this matter, due to the small number of chimeric cells present before transplantation.”

The Dutch group also investigated the role of chimeric cells in systemic lupus erythematosus (Kremer Hovinga *et al*, 2006), and reviewed the current hypotheses on the role of chimeric cells in autoimmune disease, highlighting the different pathogenic pathways by which chimeric cells might initiate detrimental immune reactions. Conversely, others have hypothesized that fetal stem cells in maternal marrow could act as a long-term reservoir of stem cells, which might repair damaged cells and might even explain why women live longer than men (O'Donoghue *et al*, 2004). “At this moment, there are more questions than answers, but there are many intriguing hypotheses that warrant further investigation,” Koopmans said.

While researchers work to assess its clinical significance and prevalence, forensic scientists have begun to take greater note of chimerism, as various prominent cases have shown. Karen Keegan from Boston, MA, USA, was hoping that one of her three sons might be a match for a kidney transplant, after her first transplant failed. Initial tests indicated that the three men are brothers, but that two of them are not her sons. “At the time, the referring nephrologist reported that she had excluded Keegan as the mother of two of her kids, and how could that be? We thought there was a problem with testing; that there was a mistake. So we had her retested and examined and sure enough it showed the same thing,” said Lynne Uhl, a transfusion medicine specialist at Beth Israel Deaconess Medical Center in Boston, who was part of the team that discovered Keegan's chimerism. “We knew this woman very well and knew without any doubt that she was the mother of these kids. That prompted us to look more carefully for an explanation for this finding.” The doctors began examining other tissues, including hair follicles and cheek swabs, and recovered stored tissue samples from several earlier minor surgical procedures.

“We were able to establish that [Keegan] carried both the HLA [human leukocyte antigen] type that was identified in her blood, and in some of her tissues the HLA type that was found in her sons and brothers. So in some of her tissues she actually had evidence for not just two HLA types but four,” Uhl said. “We hypothesized that two eggs were fertilized and very early on fused together. It wasn't that there actually was a twin that went fairly far



along in development then was resorbed, but more likely that very early on the two fertilized eggs fused together."

Lawyers in a welfare fraud case in Washington heard about the Keegan case and invited the Boston researchers to check out Lydia Fairchild. DNA testing showed that Fairchild's boyfriend had fathered her children, but ruled her out as the mother. The Boston team, which is now on another case, showed that Fairchild was in fact the mother—all her children came from a second line of chimeric cells.

Boklage commented that chimerism could have an even more important role in criminal cases. "There are guys out there escaping parental or criminal responsibilities because of standard lab testing that calls them 'not a match'. If you have a second cell line and leave behind a sperm sample that came from that line, when they come to accuse you of the rape, the DNA says you're free," he said.

Chimerism even made it into the sports news when US Olympic gold medal cyclist Tyler Hamilton fought his suspension from the sport for allegedly doping with blood from another person. Hamilton's medal from the 2004 Athens Olympics was challenged after one blood sample showed signs of transfusion, but when his second blood sample—and any chance of confirming the result—was inadvertently destroyed, the International Olympic Committee allowed him to keep the medal. Around the same time, testing for the Vuelta a España cycling race found mixed red blood cell populations in Hamilton's samples. The US Anti-Doping Agency, an independent group that enforces the World Anti-Doping Code in the USA, pursued Hamilton for allegedly transfusing himself to boost his endurance.

Hamilton fought back with a chimeric defence. David Housman, a molecular biologist at the Massachusetts Institute of Technology (Cambridge, MA, USA), read about the case and thought that the Vuelta a España testing was faulty because they "hadn't done any of the controls for sensitivity or specificity". Housman then offered Hamilton his services: "I said I'd be glad to help because I think the science here is incorrect." Among others, Housman referred to a paper by Dutch researchers, who argue that chimerism is common, affecting up to 30% of the population (van Dijk *et al*, 1996).

But the US Anti-Doping Agency's expert, Ross Brown, an Australian scientist from the team that developed the blood transfusion test used to analyse Hamilton's samples, disputed the study as an "isolated paper in the literature". Brown said it was highly unlikely that, 34 years after his birth, Hamilton would have mixed red blood cells on one histogram and not on others. He argued that the vanishing twin phenomenon in Hamilton's case was "extremely remote" and not likely to have caused the mixed red blood cell population (American Arbitration Association, 2005).

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Brown added that only about 100 cases of chimerism have been reported in the medical literature and that the American Red Cross has observed only a single case from testing millions of samples over the past 20 years. The North American branch of the international Court of Arbitration for Sport, which reviewed the Hamilton case, asked: "In light of this evidence, is Tyler Hamilton [being] the one hundred and first known case a reasonably probable explanation of [Hamilton's] histogram?" (American Arbitration Association, 2005). The panel concluded it was not and ruled that Hamilton's test results were caused by a homologous blood transfusion, not chimerism. By a 2:1 vote, they issued him with a minimum, first-offence ban from cycling for two years. An international tribunal dismissed his appeal in February 2006.

Boklage, who was not involved in the case, believes that the testing on Hamilton was incomplete. "The question can be answered by looking for those extra markers in one or more of his family members, first choice being his mother. If he is a chimera as he claims, the extra genes must belong to a sibling cell line [which] can be detected or rejected in forensic testing," he explained. Meanwhile, the Spanish newspaper *El País* reported that an official investigation in Spain revealed that Hamilton had paid a doctor to administer blood doping and other prohibited agents (Arribas & Hernandez, 2006).

Beyond the glare of the TV cameras, "[chimerism] is important and worthy of study. We don't know what effects it has on development," Boklage maintained. As it establishes two-body symmetries within a single mass of cells, chimerism could be one cause of the excess among twins of unusual brain-function asymmetry and common symmetry malformations, such as neural tube defects, congenital heart defects and orofacial clefting (Boklage, 2006). The existence of two different sets of cells in an embryo might have other profound implications on how chimeras develop and might therefore help to answer more general questions about human biology. "Are there ways that these people are different that we haven't seen?" Boklage asked. "Chimerism opens up a whole world of questions about early human development." And the questions might delve further: "Philosophically, how many souls does a chimera have?"

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